

Amendments to the Specification

These amendments insert sequence identifiers into the specification where appropriate. The undersigned hereby verifies that these amendments do not introduce new matter into the specification.

The page and line numbers correspond to the originally filed specification.

At the indicated page and line number, please replace the existing paragraphs with the following paragraphs:

(Page 3, line 23)

Indeed, to date 116 transferases have been identified in plants (examples include anthranilate N-hydroxycinnamoyl/benzoyl transferase, that catalyses the first committed reaction of phytoalexin biosynthesis, deacetylvindoline 4-O-acetyltransferase and trichothecene 3-O-acetyltransferase). Nevertheless, apart from the DFGWG (SEQ ID NO: 16) sequence motif, there are only few conserved amino acids within the transferase family. Even within the subgroup of anthranilate N-hydroxycinnamoyl/benzoyl transferase the amino acid sequences differ so much that the enzymes from Arabidopsis and clove pink are not closely related (Figure 4). This suggests that it is very difficult to correlate the function of a transferase with the sequence information provided. Hence, cloning a transferase of known function is far from straightforward.

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To isolate the full-length cDNA of HQT from tomato RT-PCR was carried out using the oligonucleotide B26: 5'-GACTCGAGTCGACATCGA(dT)17-3' (SEQ ID NO: 5) for the RT reaction (FROHMAN et al., 1988), and the oligonucleotides EST tom U: 5'-CCATGGGAAGTGAAAAAATGATGAAAA TTAATATC-3' (SEQ ID NO: 6) (corresponding to nucleotides 128 to 160 of the tomato EST clone EST263250 and introducing a NcoI-site in front of the ATG) and EST tom L: 5'- GGATCCTCATAATTCATATAAATATTTTCAAATA-3'

(SEQ ID NO: 7) (corresponding to nucleotides 472 to 500 of the tomato EST clone EST268373 and introducing a BamHI-site after the Stop-Codon) for the PCR reaction. The EST clones were identified by BLAST search using the peptide sequences obtained from Q-TOF analysis of the purified enzyme.

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The isolation of the cDNA of HQT from tobacco was conducted in two steps:

by 3'-RACE to amplify the C-terminal part of the cDNA using the oligonucleotide B26: 5'- GACTCGAGTCGACATCGA(dT)17-3' (SEQ ID NO: 5) for the RT reaction, and the oligonucleotides EST tob inter: 5'- GAGCACGTCGAGTATCATCCTCCTC CATC-3' (SEQ ID NO: 8) (corresponding to nucleotides 6 to 34 of the tobacco EST clone AB001550), the nested oligonucleotide EST tob inter2 U: 5'- ctaatttcacatcaaaaagccttagaatccac-3' (SEQ ID NO: 9) (corresponding to nucleotides 36 to 66 of the tobacco EST clone AB001550 and introducing a Hind III-site) and B25: 5'- GACTCGAGTCGACATCG-3' (SEQ ID NO: 10) for the PCR reaction; by RT-PCR to amplify the N-terminal part of the cDNA using the oligonucleotide B26: 5'- GACTCGAGTCGACATCGA(dT)17-3' (SEQ ID NO: 5) for the RT reaction, and the oligonucleotides EST tom U and EST tob inter2 L: 5'- GTGGATTCTAAGCTTTTGATGATGAAATTAG-3' (SEQ ID NO: 11) (corresponding to nucleotides 36 to 66 of the tobacco EST clone AB001550 and introducing a Hind III-site) for the PCR reaction.

5 µg total RNA isolated from tobacco leaves were used for the RT reaction which was carried out using the SUPERSRIPT™ First-Strand Synthesis System according to the manufacturers (GIBCO BRL) instructions.

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For gene silencing of HQT, to obtain iRNA constructs 600 bp cDNA-fragments of HQT from tobacco and tomato were amplified by PCR using the oligonucleotides G-tom sil U: 5' -

GGGGACAAGTTTGTACAAAAAAGCAGGCTCAGATTACTCGTTTCAAGTGTGGC -3' (SEQ ID NO: 12), G-tom sil L: 5' -
GGGGACCACTTTGTACAAGAAAGCTGGGTTGATAA ATCAGGTTGT AATTCGAGG -
3' (SEQ ID NO: 13) (corresponding to the nucleotides 424 to 447 and 1020 to 1044 of the tomato cDNA clone) and G-tob sil U: 5' -
GGGGACAAGTTTGTACAAAAAAGCAGGCTCAAATGTGGTGGAGTTTCACTG - 3' (SEQ ID NO: 14), G-tob sil L: 5' -
GGGGACCACTTTGTACAAGAAAGCTGGGTCGGGCAGT AATTCGAGGTAATC (SEQ ID NO: 15) (corresponding to the nucleotides 438 to 460 and 1034 to 1056 of the tobacco cDNA clone). The cloning of the PCR fragments into the vector pDONR201 was carried out using the Gateway™ Cloning system, according to the procedures provided by the manufacturers (Invitrogen). After sequencing the fragments were cloned in both directions into the RNAi plasmid pFRN (kindly provided by M. Denekamp), which contains attR recombination site in sense and antisense orientation spaced by an intron to enable the formation of double stranded RNA. The production of dsRNA is driven by the 35S promoter. The constructs pFRN-tom and pFRN-tob were used for stable and transient transformation of tomato and tobacco plants.

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Figure 3 is an alignment of HQT from tobacco and tomato using MultiAlin (Corpet), 1988). (SEQ ID NOS: 16, 17, 18, 19, 20, 21, 22, 23) Black background represents highly conserved regions of identical or similar amino acid residues, while they grey background indicates less conserved regions.